Activation of metabotropic glutamate receptor type 2/3 suppresses transmission at rat hippocampal mossy fibre synapses

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1. The effects of metabotropic glutamate receptor (mGluR) agonists on excitatory transmission at mossy fibre–CA3 synapses were studied in rat hippocampal slice preparations using both extracellular and whole-cell clamp recording techniques.

2. Application of a novel and potent mGluR2/mGluR3-specific agonist (2*S*,1′*R*,2′*R*,3′*R*)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV, 0·1 μM) reversibly suppressed field excitatory postsynaptic potentials evoked by mossy fibre stimulation. DCG-IV at the same concentration did not affect other glutamatergic excitatory transmissions at the commissural/associational input to CA3 or at the Schaffer collateral/commissural input to CA1 regions.

3. This suppressing effect of DCG-IV on mossy fibre transmission was dose dependent and partly antagonized by a competitive mGluR antagonist (+)-methyl-4-carboxylphenylglycine (1 mM).

4. The field potential changes induced by pressure application of glutamate (0·1 mM) to the stratum lucidum of the CA3 region was unaffected by 0·1 μM DCG-IV.

5. In whole-cell clamp experiments, 0·1 μM DCG-IV suppressed excitatory postsynaptic currents evoked by mossy fibre stimulation without inducing detectable inward current in CA3 neurons, and paired-pulse facilitation was enhanced by DCG-IV application.

6. These results suggest that mGluR2/mGluR3 are specifically expressed at mossy fibre synapses in the hippocampal CA3 region, and activation of the receptor suppresses synaptic transmission by an action on a presynaptic site.

A family of metabotropic glutamate receptors was revealed by recent molecular cloning studies (Nakanishi, 1994), and at least eight subtypes, termed mGluR1–mGluR8, have been identified so far (Masu, Tanabe, Tsuchida, Shigemoto & Nakanishi, 1991; Abe, Sugihara, Nawa, Shigemoto, Mizuno & Nakanishi, 1992; Tanabe, Masu, Ishii, Shigemoto & Nakanishi, 1992; Nakajima *et al.* 1993; Tanabe, Nomura, Masu, Shigemoto, Mizuno & Nakanishi, 1993; Okamoto *et al.* 1994; Duvoisin, Zhang & Ramonell, 1995). While sharing sequence similarities, these subtypes exhibit different agonist selectivities, signal transduction mechanisms and distributions in the brain. To elucidate the functional significance of each subtype, two kinds of experimental approach have been used. One is a genetic approach to generate the 'knock-out' mice of a certain mGluR subtype by a gene-targeting technique, and has successfully shown the involvement of mGluR1 in certain forms of synaptic and behavioural plasticities (Aiba, Chen, Herrup, Rosenmund, Stevens & Tonegawa, 1994*a*; Aiba *et al.* 1994*b*; Conquet *et al.* 1994). However, destruction of the gene in embryonic stem cells may cause compensation with other genes during development, and some complementary approach is needed to determine the physiological functions of mGluR subtypes. The other is a pharmacological approach to synthesize subtype-specific agonists or antagonists, although such pharmacological tools have been severely limited to date. The potent mGluR2/mGluR3-selective agonist (2*S*,1′*R*,2′*R*,3′*R*)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV) is one of such subtype-selective drugs, and has been shown to have potent suppressing actions on both excitatory (Ishida, Saitoh, Shimamoto, Ohfune & Shinozaki, 1993; Lovinger & McCool, 1995) and inhibitory synaptic

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transmission (Hayashi *et al.* 1993; Poncer, Shinozaki & Miles, 1995) in the central nervous system. In the present report, we describe a marked suppressing action of DCG-IV on the excitatory transmission at mossy fibre synapses in the hippocampal CA3 region, and the site of this action (pre- *vs.* postsynaptic) is discussed.

Part of the present work has been reported elsewhere in an abstract form (Kamiya, Sawada, Yoshino & Yamamoto, 1995).

METHODS

Wistar rats (20–30 days old) were anaesthetized with ether, and one hippocampus was quickly removed and immersed in an ice-cold oxygenated standard solution composed of (mM): NaCl, 127; KCl, 1·5; KH_2PO_4, 1·24; $MgSO_4$, 1·3; $CaCl_2$, 2·4; $NaHCO_3$, 26; and glucose, 10. The solution was saturated with 95% O_2 and 5% CO_2. Transverse sections of the hippocampus (0·3–0·4 mm thick) were prepared using standard techniques as previously described (Yamamoto, 1972). They were incubated in the standard solution at 32 °C for at least 40 min and transferred one by one into an observation chamber. All recordings were made at room temperature (24–28 °C) in the chamber which was continuously perfused at a rate of 3 ml min^{-1}.

Electrical stimuli were delivered at a frequency of 0·1 Hz through a stimulating electrode composed of a pair of stainless-steel wires insulated except for the tips and fixed side by side so that a pole protruded from the other pole by about 0·7 mm. All stimuli were diphasic constant-current pulses with initial negativity at the protruded cathodal pole. To stimulate the mossy fibre pathway, the cathodal pole was inserted into the stratum granulosum of the dentate gyrus, and extracellular field excitatory postsynaptic potentials (fEPSPs) were recorded in the stratum lucidum of the CA3 region with glass microelectrodes of about 10 μm tip diameter filled with 0·9% NaCl. To record commissural/associational (Co/A) fEPSPs in the CA3 region, stimulating and recording electrodes were inserted in the stratum radiatum of the CA3 region. To record Schaffer collateral/commissural (SCC) fEPSPs, both electrodes were inserted into the stratum radiatum of the CA1 region. In some experiments, the responsiveness of postsynaptic neurons to a brief pulse of L-glutamate (Glu) was examined as follows. A recording glass microelectrode of about 10 μm tip diameter filled with the standard solution containing 0·1 mM Glu was connected to a pressure-ejection device (BH-2, Medical Systems Corporation, Great Neck, NY, USA) and inserted into the stratum lucidum where the most proximal portion of apical dendrites of CA3 neurons exist. Glu was ejected from the microelectrode by brief pressure pulses (20–40 ms duration, 0·15–0·25 kg cm^{-2}) and resultant field potential changes, which probably reflected excitation of CA3 neurons near the ejection site, were recorded through the same microelectrode. In separate experiments, voltage-clamp recordings were made from CA3 neurons using a 'blind' whole-cell patch-clamp technique (Blanton, Lo Turco & Kriegstein, 1989). Patch pipettes were filled with an internal solution (pH 7·3) containing (mM): potassium-gluconate, 115; KCl, 15; $MgCl_2$, 6; EGTA, 0·2; Hepes, 10; KOH, 13; Na_2ATP, 5. When filled with this solution patch pipettes had a resistance of 3–8 MΩ. Cells were voltage clamped at −70 mV.

Field potentials and whole-cell currents were amplified with a microelectrode amplifier (MEZ-8201, Nihon Kohden, Tokyo, Japan) and a patch-clamp amplifier (CEZ-2200, Nihon Kohden), respectively, and stored on either an FM tape-recorder (A-47, Sony Magnescale, Tokyo, Japan) or digital audio tape-recorder (DTR-1200, Biologic, Echirolles, France) for further off-line analysis using in-house data-analysing software. Averaged data from different experiments are presented as means ± S.E.M. Statistical analysis was performed using Student's paired *t* test, and $P < 0·05$ was accepted for statistical significance.

Drugs used in this study included 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), D(−)-2-aminophosphonopentanoic acid (D-AP5), (+)-methyl-4-carboxyphenylglycine (MCPG; all from Tocris Cookson, Bristol, UK), DL-2-amino-4-phosphonobutyric acid (AP4, Calbiochem) and (2*S*,1′*R*,2′*R*,3′*R*)-2-(2,3-dicarboxycyclo-propyl) glycine (DCG-IV; Ishida *et al.* 1993).

RESULTS

Selective suppression of mossy fibre fEPSPs by DCG-IV

fEPSPs evoked by mossy fibre stimulation were recorded in the stratum lucidum in the CA3 region and the effects of several mGluR agonists were examined. Application of a mGluR agonist 1*S*,3*R*-ACPD (5 μM) and a mGluR2/3 agonist DCG-IV (0·1 μM) reversibly reduced the fEPSP amplitudes to 31·3 ± 3·6 and 19·0 ± 3·8% ($n = 6$) of those before drug application, respectively (Fig. 1*A*). A mGluR4/6/7/8-selective agonist AP4 (50 μM: Nakajima *et al.* 1993; Tanabe *et al.* 1993; Okamoto *et al.* 1994; Duvoisin *et al.* 1995) weakly suppressed the fEPSPs (to 75·0 ± 2·0%). At the end of each experiment, a mixture of 10 μM CNQX (non-NMDA receptor antagonist) and 25 μM D-AP5 (NMDA receptor antagonist) was applied and almost completely abolished fEPSPs (Fig. 1*A*) without affecting presynaptic fibre volley potentials (see Fig. 3). All illustrated traces in Fig. 1 show averages of ten successive traces after subtraction of the averaged traces recorded in the presence of CNQX and D-AP5. For quantitative analysis of synaptic transmission, fEPSP amplitudes, rather than slopes of the traces after subtraction of presynaptic volley potentials, were measured throughout this study. The slope might not be a correct measure in the case of mossy fibre responses, because it was pointed out that rising phases of averaged compound mossy fibre excitatory postsynaptic currents (EPSCs) could be significantly different from those of unitary EPSCs by asynchrony of mossy fibre inputs and unknown mechanisms (Langdon, Johnson & Barrionuevo, 1993).

An examination was then made of the effect of mGluR agonists on other glutamatergic excitatory transmissions in the hippocampus. Co/A fibre input to the CA3 region makes excitatory synapses at a more distal portion of the apical dendrites of CA3 pyramidal neurons (Williams & Johnston, 1991). fEPSPs by Co/A stimulation were recorded from the synaptic region (stratum radiatum). These fEPSPs were little suppressed by 0·1 μM DCG-IV (to 97·4 ± 2·3%) but suppressed weakly by 5 μM 1*S*,3*R*-ACPD (to 83·5 ± 3·3%, $n = 4$; Fig. 1*B*). The Co/A fEPSPs in the CA3 region were

almost completely abolished by CNQX and D-AP5. The difference in DCG-IV action on two different glutamatergic synapses converging on the same group of CA3 pyramidal neurons (mossy fibre synapse *vs.* Co/A synapse) suggests that mGluR2/3 are specifically expressed at mossy fibre synapses in the CA3 region.

The SCC pathway is composed of the axons of CA3 pyramidal neurons and makes glutamatergic synapses on apical dendrites of CA1 pyramidal neurons. fEPSPs evoked by SCC stimulation were recorded from the stratum radiatum of the CA1 region. These fEPSPs were little suppressed by 0·1 μM DCG-IV (to 92·1 ± 1·0%) or by 5 μM

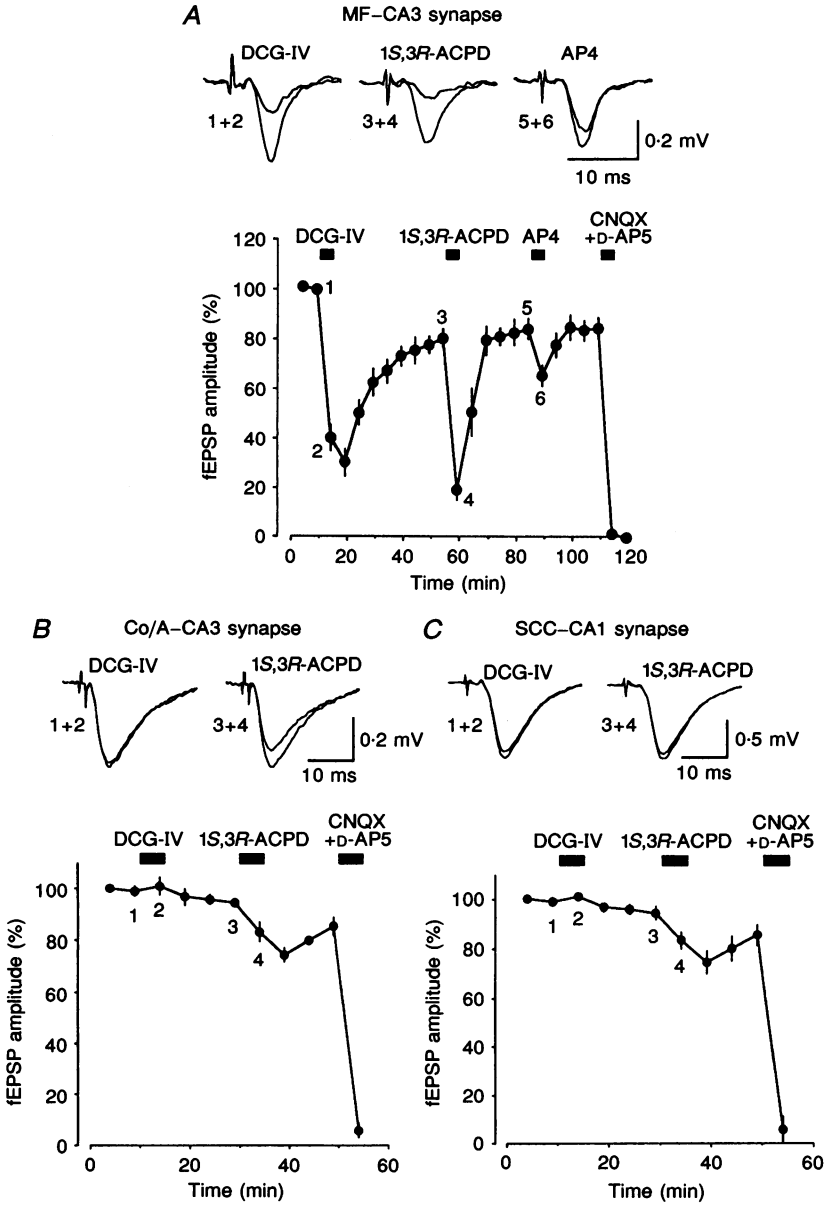

Figure 1. Selective inhibition of mossy fibre synaptic responses by DCG-IV

A, application of 0·1 μM DCG-IV (mGluR2/3-specific agonist) or 5 μM 1*S*,3*R*-ACPD markedly suppressed mossy fibre fEPSPs in rat hippocampal slices, while 50 μM AP4 (mGluR4/6/7/8-selective agonist) caused weak suppression. To stimulate mossy fibre pathways, a stimulating electrode was inserted into the stratum granulosum of the dentate gyrus, and fEPSPs were recorded from the stratum lucidum of the CA3 region. The numbers in the specimen records correspond to the times indicated in the graph with the same numbers. *B* and *C*, DCG-IV (0·1 μM) did not affect Co/A–CA3 fEPSPs (*B*) or SCC–CA1 fEPSPs (*C*) significantly, and 1*S*,3*R*-ACPD (5 μM) caused weak depression at both Co/A–CA3 and SCC–CA1 synapses. To record Co/A fEPSPs in CA3, stimulating and recording electrodes were inserted into the stratum radiatum of the CA3 region. To record SCC fEPSPs in CA1, both electrodes were inserted into the stratum radiatum of the CA1 region. All traces in this figure show averages of 10 successive traces, after subtraction of presynaptic fibre volleys and stimulus artifacts, recorded in the presence of CNQX and D-AP5.

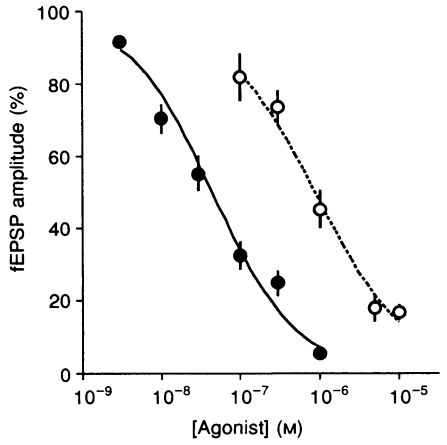

Figure 2. Dose-dependent suppression of mossy fibre fEPSPs by DCG-IV or 1*S*,3*R*-ACPD

Effect of increasing concentrations of DCG-IV (●) or 1*S*,3*R*-ACPD (○) on mossy fibre fEPSP amplitudes. Each point represents mean ± S.E.M. of 3–8 measurements, expressed as a percentage of control responses. Each drug was applied for 5 min. The data were fitted by a Hill equation with the least-squares method which gave an IC_{50} of 44 nM and a Hill coefficient of 0·81 for DCG-IV (continuous line), and an IC_{50} of 0·89 μM and a Hill coefficient of 0·74 for 1*S*,3*R*-ACPD (dashed line).

1*S*,3*R*-ACPD (to 89·4 ± 1·6%, $n = 4$; Fig. 1*C*), although a higher concentration of 1*S*,3*R*-ACPD (50 μM) has been reported to suppress these fEPSPs to approximately 50% of control (Chinestra, Anikszteyn, Diabira & Ben-Ari, 1993; see also Baskys & Malenka, 1991). This result again suggests the specific expression of mGluR2/3 at mossy fibre–CA3 synapses in the hippocampus.

Dose-dependent suppression of mossy fibre fEPSPs by DCG-IV

Next we examined the effect of DCG-IV on mossy fibre fEPSPs at different doses (Fig. 2). When applied at high doses (1–3 μM), DCG-IV almost completely abolished the fEPSPs (5·7 ± 1·3% of control at 1 μM, $n = 3$; 0% of control at 3 μM, $n = 1$, not shown), while lower doses

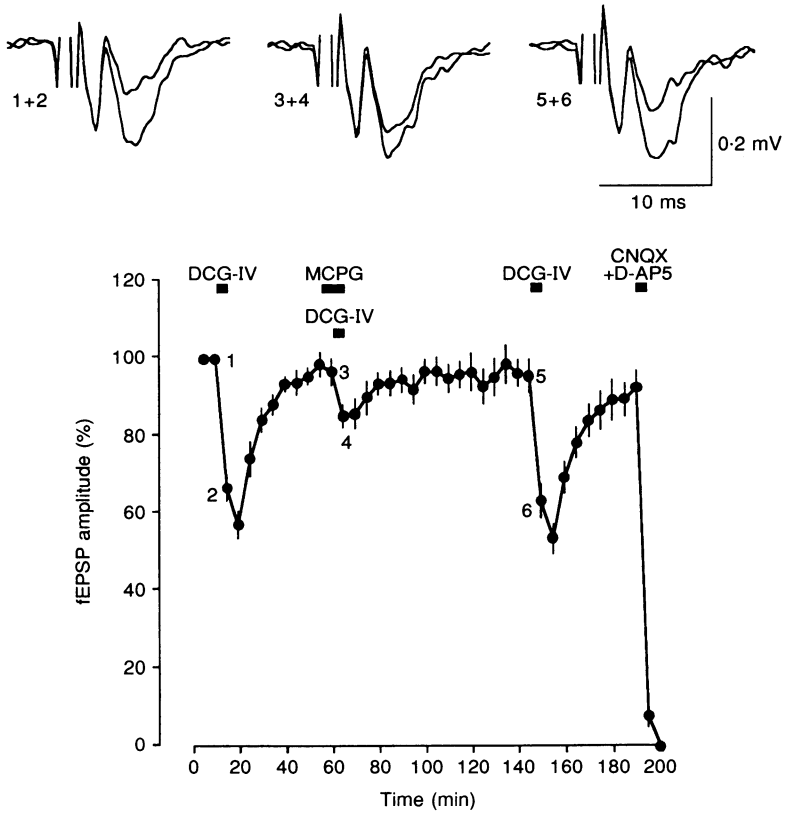

Figure 3. The suppressing action of DCG-IV on mossy fibre-induced fEPSPs was partially blocked by mGluR antagonist MCPG

Summary of 7 experiments in which DCG-IV (50 nM) was applied 3 times; first in the absence, then in the presence of MCPG (1 mM), and then again after MCPG had been washed out. Application of MCPG alone little affected mossy fibre fEPSPs while it partially reduced the effect of DCG-IV. Illustrated data traces are averages of 10 successive sweeps. The numbers in the data traces correspond to the time indicated in the lower graph. The difference between the effects of DCG-IV in the absence and presence of MCPG was statistically significant ($n = 6$; Student's t test, $P < 0·01$).

caused smaller suppression. The estimated IC_{50} value of the DCG-IV effect was 44 nM. Dose dependency of the 1*S*,3*R*-ACPD effect was also examined in a series of experiments, and the IC_{50} was estimated at 0·89 μM.

The suppressing action of DCG-IV on mossy fibre fEPSPs was partially blocked by MCPG

We tested whether the DCG-IV effect on mossy fibre transmission could be antagonized by a putative competitive mGluR antagonist MCPG (Eaton *et al.* 1993; Hayashi *et al.* 1994; Manzoni, Weisskopf & Nicoll, 1994; but see Chinestra *et al.* 1993). Application of a mixture of 50 nM DCG-IV and 1 mM MCPG for 5 min reduced the fEPSP amplitude to 88·3 ± 1·9%, while 50 nM DCG-IV alone (5 min) caused the reduction to 66·4 ± 3·2% ($n = 6$, Fig. 3). The difference was statistically significant (Student's paired t test, $P < 0·01$). MCPG at 1 mM alone did not affect the fEPSP amplitude significantly (98·2 ± 1·9% of control, $n = 6$). After prolonged washout of MCPG, the third application of DCG-IV at the same concentration (5 min) reduced the fEPSP amplitude to 65·7 ± 2·8%. This result suggests that the DCG-IV effect is antagonized by MCPG. The alternative interpretation of the result involves the possible desensitization of the DCG-IV effect on the second application. This possibility seems to be less likely, since a separate set of control experiments in which DCG-IV (0·1 μM) was applied twice with the same time course did not show clear indications of desensitization. DCG-IV at 0·1 μM suppressed fEPSPs to 45·9 ± 42 and 46·8 ± 1·5% on the first and second applications, respectively. All these results strongly suggest that the DCG-IV effect on mossy fibre responses could be antagonized by MCPG.

DCG-IV suppressed mossy fibre fEPSPs without affecting field potential changes induced by pressure application of glutamate

The suppressing action of DCG-IV on transmission at mossy fibre synapses may arise from either a decrease in transmitter release from presynaptic terminals or a decrease in postsynaptic responsiveness to transmitter, or both. To test whether the sensitivity of postsynaptic receptors is modified by DCG-IV, responses to the exogenously applied putative transmitter Glu were recorded during bath application of DCG-IV. Electrical stimuli to mossy fibres and short pressure pulses to the recording electrode (20–40 ms, 0·15–0·25 kg cm^{-2}) were applied successively, and field potentials were recorded through an extracellular glass microelectrode filled with 0·1 mM Glu dissolved in the standard solution. Application of 0·1 μM DCG-IV markedly suppressed fEPSPs evoked by mossy fibre stimulation (to 36·5 ± 2·3% of control) while responses to pressure application of Glu were almost unaffected (100·5 ± 3·1% of control, $n = 11$; Fig. 4). Both fEPSPs and glutamate responses were suppressed by a mixture of 40 μM CNQX and 25 μM D-AP5 to 5·0 ± 1·0 and 24·0 ± 3·1% of control, respectively. These results

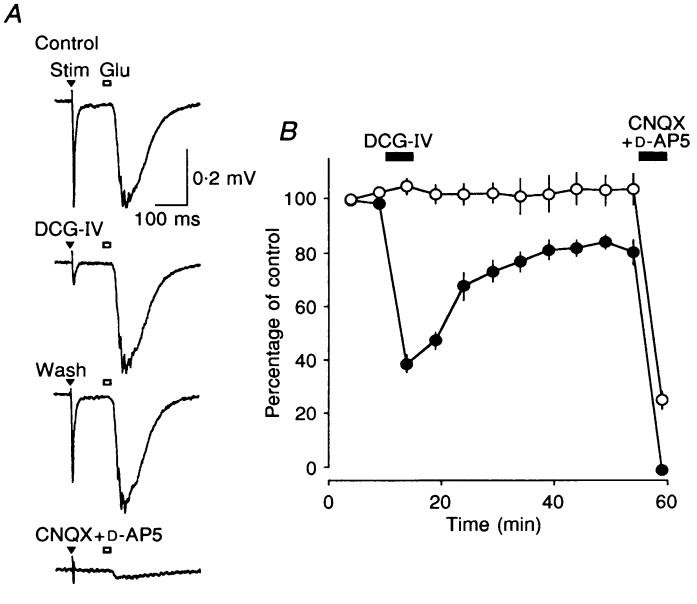

Figure 4. DCG-IV suppressed mossy fibre fEPSPs without affecting field potential changes induced by pressure application of glutamate

A, electrical stimuli (Stim) to a mossy fibre (▼) and short pressure pulses to the recording electrode filled with 0·1 mM Glu (□; 20 ms, 0·15 kg cm^{-2}) were applied successively. DCG-IV (0·1 μM) markedly suppressed the mossy fibre fEPSPs while responses to pressure application of Glu recorded through the same microelectrode were almost unaffected. Both fEPSPs and Glu responses were blocked by a mixture of 40 μM CNQX and 25 μM D-AP5, leaving only electrical or pressure stimulus artifacts and presynaptic fibre volley potentials. *B*, time course of mossy fibre fEPSP amplitudes (●) and Glu response amplitudes (○) in 11 experiments.

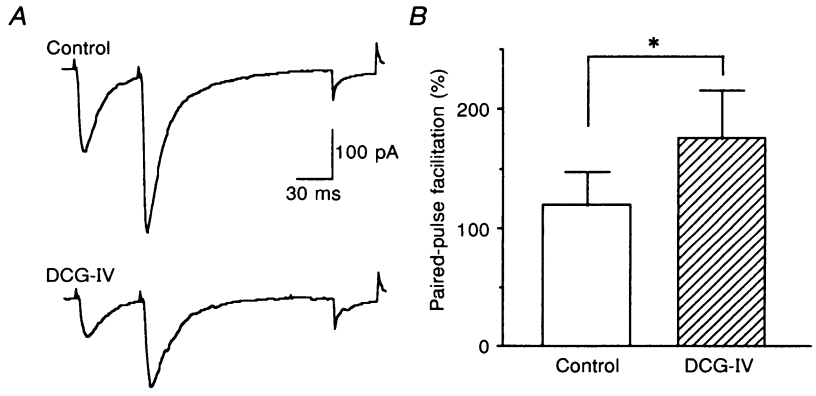

Figure 5. Paired-pulse facilitation was enhanced by DCG-IV

A, whole-cell voltage-clamp recordings from a CA3 neuron showed that DCG-IV (0·1 μM) suppressed mossy fibre EPSCs while inducing no detectable inward current. The cell was voltage clamped at −70 mV, and 2 mV hyperpolarizing voltage pulses were applied for 40 ms at the end of sweeps to monitor series resistance and input resistance. *B*, percentage facilitation of the second EPSC amplitude in response to paired stimuli with a 60 ms interstimulus interval (paired-pulse facilitation; 100% indicates that the second response is twice as large as the first response in this figure) was enhanced by 0·1 μM DCG-IV ($n = 6$; Student's t test, * $P < 0{\cdot}05$).

suggest that postsynaptic responsiveness at the mossy fibre–CA3 synapse is not affected by DCG-IV application.

Paired-pulse facilitation is enhanced by DCG-IV

An alternative method to determine whether the receptor is localized pre- or postsynaptically involves examining the effects of DCG-IV on paired-pulse facilitation (Manabe, Wyllie, Perkel & Nicoll, 1993). For this purpose, we adopted whole-cell voltage-clamp recording of mossy fibre-evoked EPSCs rather than extracellular field potential recording. In field potential recording, larger EPSP amplitudes in response to the second pulse may result in a decrease in driving force and non-linear summation (Martin, 1955) which inhibit the full expression of the paired-pulse facilitation, or result in contamination of the action potential component generated in the cell body at the stratum pyramidale. These difficulties can be avoided by whole-cell voltage-clamp recordings from CA3 pyramidal neurons. Since mossy fibres make synaptic contacts at proximal portions of apical dendrites, voltage is expected to be controlled effectively by whole-cell clamp recordings at the soma (Johnston & Brown, 1983) and inaccuracy due to non-linear summation can be minimized. DCG-IV at 0·1 μM suppressed mossy fibre EPSCs to 39·6 ± 4·9% of control while inducing no detectable inward current (0·2 ± 5·2 pA, $n = 6$) and input resistance did not change significantly (212 ± 60 and 215 ± 30 MΩ in the absence and presence of DCG-IV, respectively, $n = 6$; Student's t test, $P > 0{\cdot}05$) when CA3 neurons were voltage clamped at −70 mV (Fig. 5*A*). The percentage facilitation of the second EPSC amplitude in response to paired stimuli with a 60 ms interstimulus interval (paired-pulse facilitation) was enhanced by 0·1 μM DCG-IV (119 ± 29 and 176 ± 40% in the absence and presence of DCG-IV, respectively; $n = 6$; Student's t test, $P < 0{\cdot}05$; Fig. 5*B*). In contrast, a low concentration of CNQX (0·5 μM) which was expected to suppress the synaptic transmission post-synaptically did not alter paired-pulse facilitation significantly (143 ± 55 and 125 ± 34% in the absence and presence of 0·5 μM CNQX, respectively; $n = 6$; Student's t test, $P > 0{\cdot}05$). These results are consistent with the idea that DCG-IV might act presynaptically to suppress transmitter release from mossy fibre terminals in the hippocampal CA3 region.

DISCUSSION

The present study demonstrates that a potent subtype-selective metabotropic glutamate receptor agonist DCG-IV strongly suppresses excitatory transmission at mossy fibre–CA3 synapses, but not at Co/A–CA3, or at SCC–CA1 synapses, in rat hippocampal slices. These findings suggest that metabotropic glutamate receptors types 2/3 (mGluR2/mGluR3) are specifically expressed at mossy fibre–CA3 synapses in the hippocampus. The suppressing action of DCG-IV on mossy fibre–CA3 synapses was not due to an indirect postsynaptic effect (e.g. depolarization), or to direct interaction with postsynaptic ionotropic glutamate receptors (mainly non-NMDA receptors at this synapse).

Heterogeneous distribution of mGluR subtypes in the hippocampus has been suggested by previous studies using *in situ* hybridization analysis. In the hippocampus, mRNA encoding mGluR2 or mGluR3 is only expressed in granule cells of the dentate gyrus, and is not detected in CA3

pyramidal cells (Tanabe *et al.* 1992, 1993). Since mossy fibres are the axons of the granule cells in the dentate gyrus, it seems likely that mossy fibre terminals specifically express mGluR2/mGluR3.

In this study, electrical stimuli were delivered through an extracellular stimulating electrode inserted into the stratum granulosum of the dentate gyrus. Therefore the changes in excitability of the cell bodies of granule cells or their axons (mossy fibres) may cause suppression of mossy fibre fEPSPs without changes in postsynaptic cells. However, the absence of changes in presynaptic fibre volley potentials argues against this possibility.

DCG-IV at higher concentrations than 10 μM has been shown to activate NMDA receptors and depolarize rat spinal cord neurons (Ishida *et al.* 1993). The DCG-IV concentration used in most experiments of this study (0·1 μM) was much lower than 10 μM, and this lower concentration does not cause detectable inward current in CA3 neurons under voltage clamp (holding potential, −70 mV). A direct test to examine changes in postsynaptic responsiveness by using simultaneous recording of both fEPSPs and Glu-evoked field potentials also failed to show any changes in postsynaptic responsiveness. These findings suggest that DCG-IV does not affect postsynaptic CA3 pyramidal neurons, and its suppressing action seems to involve activation of presynaptic mGluR2/mGluR3 on mossy fibre terminals.

A presynaptic site of action for DCG-IV was also suggested by the increase in paired-pulse facilitation. We adopted to examine the effect of DCG-IV on paired-pulse facilitation, rather than miniature EPSCs (mEPSCs), in this study. Although the analysis of mEPSCs is useful to quantify a presynaptic *vs.* postsynaptic effect in general, the interpretation of the mEPSCs analysis seems to be complicated in this case. CA3 pyramidal cells receive at least two major glutamatergic inputs, namely mossy fibre and Co/A fibre inputs. The EPSPs at these two inputs exhibit differential sensitivities to DCG-IV (Fig. 1). Since it is not possible to distinguish the origins of the observed mEPSCs (i.e. mossy mEPSCs or Co/A mEPSCs), heterogeneous sensitivities to DCG-IV may hamper quantitative interpretation of the results.

The response to mossy fibre stimulation could be contaminated by responses activated by disynaptic activation of associational fibres and/or associational axon reflex input (Weisskopf & Nicoll, 1995). Therefore, some percentage of the observed inhibition may actually occur at such synapses. However, contribution of inhibition at CA3 associational synapses might be small, if any, as expected from the observation that DCG-IV barely suppressed Co/A synapses in the CA3 region (Fig. 1*B*).

It must also be noted that the potency of 1*S*,3*R*-ACPD at the presynaptic receptor might be somewhat overestimated due to a contribution of a postsynaptic effect. This agonist has been reported to depolarize hippocampal pyramidal neurons (Guérineau, Gähwiler & Gerber, 1994), and this postsynaptic effect might suppress transmission by reducing the driving force for ion permeation through postsynaptic glutamate receptor–channels.

An mGluR4/6/7/8-selective agonist AP4 at 50 μM suppressed rat mossy fibre response weakly (by about 25% of control) in this study, while it was reported that AP4 at 200 μM did not affect rat mossy fibre response significantly (by 7 ± 5% of control; Lanthorn, Ganong & Cotman, 1984). This apparent discrepancy may arise from the different ages of the animals used. Relatively young animals were used in this study (20–30 days) while Lanthorn *et al.* used adult rats (45–60 days). In fact, the developmental change in the distribution of mGluR7, one of the AP4-sensitive subtypes which widely distributes throughout the nervous system, was recently reported (Kinzie, Saugstad, Westbrook & Segerson, 1995).

How might DCG-IV suppress transmitter release from mossy fibre terminals? Signal transduction mechanisms coupled to cloned mGluR2 and mGluR3 were investigated in CHO (Chinese hamster ovary) cells transfected to express the receptors, and were shown to be negatively coupled to forskolin-stimulated cyclic AMP formation (Tanabe *et al.* 1992, 1993). Recent findings that an increase in cyclic AMP concentration within mossy fibre terminals leads to persistent enhancement of transmitter release (Huang, Li & Kandel, 1994; Weisskopf, Castillo, Zalutsky & Nicoll, 1994) suggest that a decrease in cyclic AMP concentration is likely to be involved in this DCG-IV-induced suppression of transmitter release process.

Excitatory transmission at corticostriatal synapses is suppressed presynaptically by several mGluR agonists including DCG-IV (Lovinger & McCool, 1995), and this presynaptic depression by mGluR activation is inhibited by pretreatment with protein kinase C activator phorbol esters (Swartz, Merritt, Bean & Lovinger, 1993). Therefore, DCG-IV-sensitive subtype(s) (mGluR2/mGluR3) responsible for presynaptic depression in this synapse could also be regulated with phosphorylation by protein kinase C. Signal transduction mechanisms responsible for the DCG-IV effect on hippocampal mossy fibre synapses, including such a 'cross-talk' between several second messengers, have to be determined in future investigations.

Another possible mechanism is that DCG-IV inhibits action potential-induced Ca^{2+} influx into presynaptic terminals and thereby inhibits transmitter release. Data from a neuronal heterologous expression system indicate that mGluR2 activation can inhibit N-type Ca^{2+} channels (Ikeda, Lovinger, McCool & Lewis, 1995). Since synaptic transmission at mossy fibre–CA3 synapses in the hippocampus was shown to involve N-type Ca^{2+} channel activation (Kamiya, Sawada & Yamamoto, 1988), direct downregulation of presynaptic Ca^{2+} channels seems to be

likely. Ca^{2+} influx into presynaptic terminals could also be regulated by K^+ channels which are involved in the repolarization phase of action potentials. In fact it was suggested that the presynaptic inhibitory action of a mGluR agonist in neonatal CA1 synapses involves activation of presynaptic K^+ channels (Yoshino & Kamiya, 1995).

Another important consideration of this study arises from the observation that DCG-IV application only results in a reversible depression of synaptic transmission and never results in long-term potentiation (LTP) after complete washout of this drug. Ito & Sugiyama (1991) showed that a mGluR agonist ibotenate produced LTP of mossy fibre responses. Bashir *et al.* (1993) showed that mossy fibre LTP by tetanic stimulation is blocked by a mGluR antagonist MCPG (but see Manzoni *et al.* 1994). Therefore, it seems likely that certain mGluR subtype(s) are involved in LTP in mossy fibre–CA3 synapses. Recent findings by Conquet *et al.* (1994), who showed impaired mossy fibre LTP in mGluR1 deficient 'knock-out' mice, suggest that activation of mGluR1 is essential for LTP in this synapse. Messenger RNA encoding mGluR1 is strongly detected both in presynaptic granule cells of the dentate gyrus and postsynaptic pyramidal cells of the CA3 region (Masu *et al.* 1991). Bidirectional modulation (depression *vs.* potentiation) of synaptic transmission at mossy fibre–CA3 synapses by distinct mGluR subtypes (presynaptic mGluR2/3 *vs.* pre- and/or postsynaptic mGluR1) may serve as important factors which regulate the efficacy of hippocampal neural circuitry in an activity-dependent manner.

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Acknowledgements

We thank Drs T. Manabe and T. Ohno-Shosaku for technical advice. This work was supported by Grant-in-Aid No. 07279216 for Scientific Research on Priority Areas on 'Functional Development of Neural Circuits', and No. 07780698 the Ministry of Education, Science and Culture of Japan.

Received 18 August 1995; accepted 10 January 1996.